W1 Workshop Abstracts

PICES XIV W1-2445 Oral

Paralytic shellfish toxins from *Alexandrium* in Puget Sound, Washington, U.S.A.

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The first illnesses and deaths in Washington State due to paralytic shellfish poisoning were documented in the 1940s, resulting in the establishment of one of the longest monitoring programs for paralytic shellfish toxins in commercial and recreational shellfish in the U.S. An analysis of the Washington Department of Health's monitoring data allowed us to examine temporal changes in shellfish toxin levels and geographical distribution of shellfish harvesting closures. When the values of toxins in shellfish were normalized to control for variable levels of toxin accumulation in different shellfish species, the observed increase in paralytic shellfish toxin levels in Puget Sound shellfish was not due to the shift in species monitored. A geospatial map of the first shellfish closures or paralytic shellfish poisoning event in each Puget Sound basin suggests that over time, toxigenic *Alexandrium* cells have been transported from northern to southern Puget Sound, with the initial "seed" population of cells in Washington State likely originating from the inland or coastal waters of Canada. Large-scale events, such as the bloom that occurred in the Whidbey and Central basins in 1978, may have been induced by global climate changes or shifts, such as the Pacific decadal oscillation. Although greater numbers of closures have been observed over time in many basins of Puget Sound, closures as a percentage of total samples analyzed have decreased or remained constant in all basins, indicating that the Washington Department of Health has increased its margin of safety by monitoring at more sites in recent years.

PICES XIV W1-2508 Invited

Biology of the diatom *Pseudo-nitzschia*, producer of the ASP toxin domoic acid

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This talk will summarize the current knowledge about the distribution, physiology and toxicity of *Pseudo-nitzschia* species. In 1987, *P. multiseries* was discovered as the source of domoic acid (DA) that caused amnesic shellfish poisoning in eastern Canada. Since then, a total of 12 species of *Pseudo-nitzschia*, worldwide, have been shown to be DA producers. Much of what has been learned about its physiology comes from studying *Pseudo-nitzschia* isolates in the laboratory. A commonality is that DA is produced when cells are stressed; so far by silicate, phosphorus, or iron limitation, or by excess copper. The presence of certain bacteria and of selenium enhances toxicity. A wide variability in toxicity is found among isolates of the same species, and toxicity can decrease over time. Some of this can be explained by genetic variation and perhaps by temporal changes in bacterial composition. The sexual reproduction of four *Pseudo-nitzschia* species has been documented, and toxicity may also reflect the stage in the sexual cycle. This genus is found on virtually every coast, and is selected for after iron enrichment of HNLC regions of the world's oceans. The role of domoic acid is still being debated. There is no evidence that it acts as an antifeedant. Laboratory, and now field, studies are showing that its siderophore-like properties may confer an advantage by chelating trace metals. Factors that trigger toxic blooms must still be deciphered. Without a resting stage, viable cells may accumulate in thin layers, until transported to surface waters. A greater understanding is needed before toxic blooms can be predicted.

PICES XIV W1-2436 Oral

Inorganic and organic nitrogen uptake capabilities of the toxigenic diatom *Pseudo-nitzschia australis*

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The nitrogen uptake capabilities of the toxigenic diatom *Pseudo-nitzschia australis* were examined in uni-algal laboratory cultures at saturating photosynthetic photon flux densities (100 µE m^{-2} s^{-1}) and 15°C. The kinetics of nitrogen (nitrate, ammonium, urea and glutamine) uptake as a function of substrate concentration were estimated from short (10-min) incubations using the ^{15}N-tracer technique, and are compared to the long-term exponential growth rates of *P. australis* determined in semi-continuous, batch cultures grown on the various nitrogen substrates. Based on the estimated nitrogen uptake kinetic parameters, nitrate is the preferred nitrogen substrate at both saturating and sub-saturating concentrations, whereas rates of urea uptake by *P. australis* did not saturate even at concentrations as high as 36 g-at N L^{-1}. The growth rate (determined using cell abundance over time) of *P. australis* was slower for cells grown on urea (0.5 d^{-1}) compared to the cells grown on nitrate and ammonium, which both maintained significantly greater growth rates (*ca.* 0.9 d^{-1}). However, the particulate domoic acid content of the urea-grown cells was substantially greater than cells grown on either nitrate or ammonium. These results demonstrate the capability of this diatom to grow equally well on both oxidized and reduced forms of nitrogen, supporting our field observations that *P. australis* blooms during both upwelling and non-upwelling conditions off the west coast of North America. During these times, substantial differences in the nitrogenous nutrition of *P. australis* can be expected, and anthropogenic inputs of reduced N substrates could contribute significantly to its growth and toxicity.

PICES XIV W1-2266 Oral

Notes on *Pseudo-nitzschia* and *Alexandrium* bloom occurrence in Japanese coastal waters

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In Japanese coastal waters, both *Pseudo-nitzschia* and *Alexandrium* species occur. *Pseudo-nitzschia* is one of the common phytoplankton in Japanese waters. However, in spite of intensive surveys conducted by the Fisheries Agency and several universities since 1993, domoic acid contamination (excess of quarantine level) in wild and aquacultured shellfish has not been detected so far. Therefore, only a few monitoring programs are set for ASP toxins in Japanese coastal waters. In contrast, PSP toxin contamination by *Alexandrium* (*A. tamarense*, *A. catenella* and *A. tamiyavanichii*) has been widely detected and an active monitoring program has been implemented for PSP toxins in Japanese coastal waters by the regional fisheries research organizations. Among the above three *Alexandrium* species, *A. tamarense* and *A. catenella* appear over a wide area and often cause toxin contamination in shellfish. From a morphological and genetic point of view, *A. tamarense* and *A. catenella* are closely related species, but these two species usually bloom in different areas in different seasons; rarely blooming simultaneously. Studies on these two *Alexandrium* species clarified that while they have the same life cycle (cyst and vegetative stages), their ecological and physiological characteristics are different. For example, duration of the cyst dormant period varies between *A. tamarense* (several months to half year) and *A. catenella* (several days to one week). Moreover, the temperature "window" for the cyst germination differs between *A. tamarense* (7.5˚C to 20˚C) and *A. catenella* (7.5˚C to 25˚C). These differences in cyst dormancy-germination characteristics would partly lead to spatial and temporal differentiation of blooming events.

PICES XIV W1-2597 Oral

Occurrence of *Pseudo-nitzschia* and *Alexandrium* species in Korean coastal waters

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A twelve-month field survey of the potentially toxic genus *Pseudo-nitzschia* was carried out in Chinhae Bay, an area of commercial culturing and harvesting of oysters. Ten species of *Pseudo-nitzschia* were identified during the study: *Pseudo-nitzschia multiseries, P. pungens, P. multistriata, P. delicatissima, P. cuspidata, P. subfraudulenta, P. granii, P. fraudulenta, P. subpacifica*, and an unidentified *Pseudo-nitzschia* sp. *Pseudo-nitzschia pungens* had the highest cell abundance, followed by *P. delicatissima* and *P. multistriata*. Although *Pseudo-nitzschia* species varied depending on month and sampling site, most were present at higher cell densities in lower temperature waters except for *Pseudo-nitzschia multistriata*.

The motile cells of *Alexandrium tamarense*, PSP-producing species, appeared in January and reached their high concentration from March to May. Mussels became toxic in February and showed the highest level of toxicity from April to May. *Alexandrium* resting cysts were also found at every station surveyed, but the abundance was greatly variable depending on locality. Cyst collected in different seasons showed a large difference in the germination rate with showing relatively higher frequency in the cold water season; 9% (Oct.), 73% (Jan.), 68% (Apr.), and 44% (Jul.). Germination of *Alexandrium* resting cysts in Chinhae Bay was assumed to be largely controlled by the seasonal rhythmic pattern.

PICES XIV W1-2288 Oral

The distribution and HAB formation of *Pseudo-nitzschia pungens* in Chinese coastal waters

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Pseudo-nitzschia pungens in is a common and important species of phytoplankton in Chinese coastal waters. There were 5 recorded HABs caused by *Pseudo-nitzschia pungens*. In Bohai Sea, it is abundant in spring. In the Yellow Sea, high cell numbers appear in spring or early summer. High cell numbers occur in the East China Sea in early autumn or winter. In the South China Sea, *Pseudo-nitzschia pungens* is most abundant in the coast waters of Guangdong Province. Harmful blooms of *Pseudo-nitzschia pungens* usually are located south of Changjiang River (Yangtze River) Estuary. A *Pseudo-nitzschia pungens* HAB was found in upwelling area of Zhejiang Province in Aug. 1981. Two other blooms occurred in the western region of coastal water of Xiamen City in June 1987 and Dapeng Bay in Guangdong, respectively. These HAB's consisted of *Pseudo-nitzschia pungens* but also included other diatom species. It is reported that *Pseudo-nitzschia pungens* can be toxic, however, domoic acid has not been found in Chinese strains.

PICES XIV W1-2572 Oral

Microsatellite markers reveal population genetic structure of the toxic dinoflagellate *Alexandrium tamarense* (Dinophyceae) in Japanese coastal waters

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Transport of vegetative cells and resting cysts of the toxic dinoflagellate, *Alexandrium tamarense* (Lebour) Balech, which is responsible for paralytic shellfish poisoning (PSP), either in ships' ballast water or via translocation of shellfish stocks has been proposed as an explanation for its global increase. However, the extent of contribution to the apparent "cosmopolitanism" of this species could not be assessed, as techniques to distinguish unambiguously between populations were insufficient to test dispersal theories. However, recently highly polymorphic microsatellite markers have been developed to investigate the genetic structure of *A. tamarense* populations. Here we analyzed nine microsatellite loci among 10 samples along with Japanese and Korean coasts. The nine microsatellites varied widely in number of alleles and gene diversity across

populations. The analysis revealed that Nei's genetic distance correlated significantly with geographic distance in pairwise comparisons, and there was genetic differentiation in about half of 45 pairwise populations, clearly indicating genetic isolation among populations in compliance with geographic distance and restricted gene flow by natural dispersal through tidal currents among the populations. On the other hand, high P-values in Fisher's combined test were detected in 5 pairwise populations, indicating the similar genetic structure and the close relationship genetically. These findings suggest that disturbance of genetic structures of *A. tamarense* populations by human activity and gene flow by a human-assisted dispersal have occurred between these populations.

PICES XIV W1-2281 Oral
Species of the genera *Alexandrium* and *Pseudo-nitzschia* from the east coast of Russia

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Species of the genera *Alexandrium* and *Pseudo-nitzschia* from the east coast of Russia were studied by light and electron microscopy using field material and cultures. Ten *Pseudo-nitzschia* species were found: *P. pungens*, *P. multiseries*, *P. multistriata*, *P. calliantha*, *P. americana*, *P.* cf. *pseudodelicatissima*, *P. delicatissima*, *P. fraudulenta*, *P.* cf. *heimii*, and *P. seriata* f. *seriata*. The bloom-forming species were *P. pungens*, *P. multiseries, P. calliantha,* and *P. americana*, widely distributed along the Russian Pacific coast. Species *P. seriata* f. *seriata* and *P.* cf. *heimii* were observed only in Aniva Bay (the Sea of Okhotsk) at low densities. *P. delicatissima* was common but not numerous component of phytoplankton in study area. Eight species of the genus *Alexandrium* were recorded from the east coast of Russia: *A. tamarense, A. acatenella, A. catanella, A. ostenfeldii, A. insuetum, A. pseudogonyaulax, A. margalefi* and *A. tamutum*. Eight *Alexandrium* species were observed in the coastal waters of Sakhalin Island. Six species (excluding *A. catenella* and *A. tamutum)* were found in Peter the Great Bay of the Sea of Japan. Only three species *A. tamarense, A. ostenfeldii* and *A. acatenella* were observed in the coastal waters of Kamchatka and in the Bering Sea. It was shown that *A. tamarense* is dominant and the most widespread *Alexandrium* species on the Russian Pacific coast. Twenty-six clones of *Alexandrium tamarense* and two clones of *P. multiseries* and *P. calliantha* were analyzed by HPLC. All of the isolates, excluding *P. calliantha,* were found to be toxic.

PICES XIV W1-2422 Invited
Occurrence and effects of *Alexandrium* species in the environs of the North Pacific

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The distribution and occurrence of the historically significant red tide dinoflagellate, *Alexandrium* sp. will be reviewed. Using taxonomic observations, levels of PSP toxins in marine organisms and records of the health affects of consuming contaminated shellfish, I will consider the historical variability of this genus in the waters of the North Pacific ecosystem. From these geographic examples a comparison will be made to the intensively studied *Alexandrium* sp. blooms from other global locations to assess the probability that the North Pacific populations are controlled and transported through seed-bed reservoirs or through deep water lenses of cells or cysts or through transport of ballast waters. I will also review the contrasting mechanisms of species competition – survival and physiological dominance at relatively low community densities - and the variability of toxin content in both the cells and cysts, with emphasis on correlations to specific hydrodynamic features of individual North Pacific populations. Our understanding of the relationship between toxicity, toxin composition, physiological state and genotype we assist in our contribution to human health issues associated with *Alexandrium* species.

PICES XIV W1-2236 Oral

Preliminary study of *Pseudo-nitzschia* spp. in the Yangtze estuary (China)

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A four-season field survey of harmful algae including *Pseudo-nitzschia* was carried out in the Yangtze estuary (China) during 2003-2004. Three species of *Pseudo-nitzschia* were identified during the monitoring*; P. multiseries*, the first recorded in China, *P. multistriata,* the first recorded in the Yangtze estuary, and *Pseudo-nitzschia pungens,* which had been frequently detected. *Pseudo-nitzschia* spp. were distributed mainly in the outer region of the Yangtze river front, where the salinity was near 25 psu and the temperature 20°C on June. *Pseudo-nitzschia* spp. were found in highly turbid waters, even up to 802 mg/L, but cell numbers were highest in higher temperature, low turbidity waters. Among the 6 *Pseudo-nitzschia* species recorded to date in Chinese coastal waters, *P. multistriata, P. multiseries and P. pseudodelicatissima* have been documented elsewhere in the world as domoic acid producers. Mouse bioassays of mussels from the Yangtze estuary indicate the presence of the ASP toxin, but the existence of domoic acid still needs to be ascertained by HPLC analysis. A preliminary study during the 1980s found only *P. pungens* in these waters, indicating that *P. multistriata* and *P. multiseries* might be alien species in the Yangtze estuary. *Pseudo-nitzschia* spp. occurred in almost all the diluted waters of the Yangtze estuary, but also were relatively high in upwelling waters. Cell abundances were slightly higher in surface water than near the bottom. Seasonal fluctuations of *Pseudo-nitzschia* spp. indicate higher abundances in summer and autumn than in spring and winter.

PICES XIV W1-2289 Oral

The distribution and HAB formation of *Alexandrium* spp. in Chinese coastal waters

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Alexandrium tamarense and *Alexandrium catenella*, two PSP-producing species, are both common species of the genus *Alexandrium* in Chinese coastal water. PSP poisoning events caused by these two species are found in coastal waters of the world as well as in Chinese coastal waters. HABs of *Alexandrium* sp. usually occur in South China coastal waters, such as coastal waters of Fujian and Guangdong Provinces. However, these HABs have expanded northwards along the coast in recent years. There were blooms of *Alexandrium* sp. in area near Zhoushan Archipelago in the spring of 2002 and 2004, together with *Prorocentrium dentatum*. During these two HABs, cell numbers of *Alexandrium* sp. reached 10^6 cells/L. In Oct. 2004, a HAB of *Alexandrium* spp. occurred in coastal waters of Dalian City in North Yellow Sea. As *Alexandrium* spp. are toxic, these blooms are of concern to the Chinese government and scientists. Monitoring of HABs and toxicity in shellfish products therefore has been strengthened in Chinese coastal waters.

PICES XIV W1_HAB_Section-2579 Oral

Progress in the development of an international collaborative harmful algal event data base: The joint IOC-ICES-PICES HAE-DAT

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The IOC and ICES have jointly developed the Harmful Algal Event Data base HAE-DAT with the view to expand the partnership and thereby build a global harmful algal event database. To this effect PICES in 2004/05 became a full and equal partner in HAE-DAT. HAE-DAT provides a comprehensive format for reporting all types of algal events which are perceived by society as harmful. For the PICES region will additionally be recorded algal blooms which did not cause any harm. HAE-DAT is in the process of having its software platform upgraded and improved and of being developed with an associated mapping function. The progress will be presented for comments and discussion in conjunction with the evaluation of PICES experience in using the data input format.

PICES XIV W1_HAB_Section-2510 Oral

Oceanological conditions and HAB monitoring in Aniva Bay, Sea of Okhotsk during 2003

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During May-December 2003 regular water sampling was conducted at nearshore spots in Aniva Bay, Sea of Okhotsk. Seasonal dynamics of few species of harmful algae was observed with simultaneous oceanographic parameters observations. The variance of species of harmful algae regarding water masses properties, currents and methods for possible monitoring and detecting HAB events in the Aniva Bay are discussed.

PICES XIV W1_HAB_Section-2599 Oral

Participation in the Intergovernmental Oceanographic Commission's Harmful Algae Event Database (HAE-DAT): The first year of PICES involvement

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During the PICES XIII meeting in Honolulu, Hawaii, the Harmful Algal Bloom Section member countries unanimously decided to adopt the IOC database as the official PICES HAB database. The ICES database is now called the HAE-DAT IOC joint ICES/PICES database. During our HAB Section meeting on Saturday, October 1, 2005, an overall description of the PICES joint database with IOC/ICES and its goals will be discussed. Each country will report on involvement in the HAE-DAT effort that includes a description of monitoring programs in each country, what constitutes a "harmful event", the algae species currently described in the IOC database as harmful in each country, harmful levels, and "area codes" (coastline divisions into ~100-200 km sections for which frequency of HAB events are plotted.). A summary of the first year's participation in

the HAE-DAT collaboration will be given by a HAB Section representative from each country, including Dr. Kim (Korea), Dr. Trainer (U.S.A.), Dr. Watanabe (Japan), Dr. Zhu (China), Dr. Trick (Canada), and Dr. Orlova (Russia). Each presentation will detail the entry of data into the IOC web-based database for the year 2000 and subsequent years, if possible. The ease of HAE-DAT use and possible modifications required for northern Pacific Nations will be discussed. On Sunday morning, October 2, 2005, representatives from each country will work together to enter HAB data from the year 2001 into HAE-DAT at the extended HAB Section meeting.

PICES XIV W1_HAB_Section-2427 Oral

Domoic acid: The synergy of iron, copper and the toxicity of diatoms

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Diatom blooms generated by the alleviation of iron limitation in High Nitrate-Low Chlorophyll regions of the oceans often are composed of pennate diatoms of the genus *Pseudo-nitzschia*, many species of which periodically produce the potent neurotoxin domoic acid. We show that toxigenic diatoms have an inducible high affinity iron uptake capability that enables them to grow efficiently on iron complexed by strong organic ligands in seawater. This adaptive strategy to acquire iron when present at extremely low ambient concentrations requires copper and apparently domoic acid, a copper chelator whose production increases sharply when both iron and copper are limiting. Addition of either domoic acid or copper to coastal seawater improves the growth of *Pseudo-nitzschia* spp. on strongly complexed iron during deck incubation experiments with natural phytoplankton assemblages. Our findings suggest that domoic acid is a functional component of the unusual high-affinity iron acquisition system of these organisms. This system may help explain why *Pseudo-nitzschia* spp. are persistent seed populations in oceanic high-nitrate-low-chlorophyll regions, as well as in some neritic regions. Our findings also suggest that in the absence of an adequate copper supply, iron-limited natural populations of *Pseudo-nitzschia* will become increasingly toxic.

PICES XIV W1_HAB_Section-2238 Oral

Why the time of large scale HAB of *Prorocentrium* in the area south of Yangtze River Estuary changed in spring of 2005

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The area south of Yangtze River Estuary or the coastal water of Zhejiang Province of China is the most eutrophicated area in Chinese coastal water. It is the largest fishing ground and a region with frequent HAB events as well. A large scale HAB of *Prorocentrium* occurred in early May for three year from 2002 to 2004. The largest area of HAB reached 10,000 km^2 in 2004. However, there was no large HAB in early May of 2005. The preliminary analysis of cruise data showed that due to the cold winter with more precipitation, the nutrients in seawater were higher than other years, especially the concentration of silicate. The temperature of sea water in April was about 3^oC lower than other years. There was a diatom HAB in April. In early May, however, the water temperature was almost the same as usual. The nutrients were low as there was a bloom of *Skeletonema costatum* in April. It is clear that the proper environment condition for the early development of *Prorocentrium* sp. is essential for the formation of large scale HAB in this area in May. However, in late May a large scale bloom of *Karenia mikimotoi* was occurred. In early June, *Prorocentrium* bloom was found out, which was one month later than last three years.